Hypothesis

A MODEL FOR CONFORMATIONAL COUPLING OF MEMBRANE POTENTIAL AND PROTON TRANSLOCATION TO ATP SYNTHESIS AND TO ACTIVE TRANSPORT

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1. Introduction

Important theoretical considerations and experimental findings have led to suggestions that formation of a potential and/or pH gradients across a membrane may serve for transmission of energy from oxidations to the phosphorylating system in oxidative and photophosphorylation, see [1–6]. Resistance to acceptance of this concept has arisen in part from the lack of satisfactory suggestions or evidence as to how energy stored in a membrane potential or proton gradient could be used to drive ATP synthesis. Mitchell has suggested a "chemiosmotic" mechanism involving translocation across the membrane of an 'O_2^{2-}' group derived from P_i at the ATPase catalytic site, driven by specific protonation of phosphate oxygens [7]. I have recently called attention to deficiencies in such proposed chemiosmotic coupling ([8] and footnote*).

Other developments, however, point to an attractive means by which a membrane potential or proton gradient might be coupled to ATP synthesis. Experimental findings have led to the suggestion that ATP synthesis results from energy-linked conformational changes that change the binding of reactants at the catalytic site [10,11]. One prominent use of energy in oxidative phosphorylation has been identified as decreasing the binding of ATP to the catalytic site. More recent findings in my laboratory indicate that

* Publication of my criticisms [8] was accompanied by a rebuttal by Mitchell [9] that fails to meet the basic objections. Brief clarifications of his four 'answers' to my criticisms are as follows:

1) The assertion that because F_1 has an alkaline pH optimum, the activity of 'OH^- on F_o side of the active center of F_1 would be very high' is quite illogical. The pH optimum for an enzyme does not govern H^+ or OH^- activity at the catalytic site.

2) and 3) Mitchell states that his 'scheme does not assume the formation of the trinegative species $O{=}PO_3^{3-}$, as Boyer imagines.' But the appearance of such a species in fig. 1, stage III, of his paper is obviously not a product of my imagination. The contention that I overlooked his suggestion that Mg^{2+} may be close to the bound P_i in turn overlooked my statement, 'Modifications of the nature of the charged species participating in the steps of Mitchell's scheme, as suggested in his concluding remarks, do not obviate the chemical difficulties.' Thus binding to Mg^{2+} could aid formation of a trinegative species, but the highly positive Mg^{2+} would obviously hinder his suggested subsequent proton additions.

4) Mitchell in his paper stressed the formation of $-OH_2^+$ group on the phosphorus as a good leaving group. His apparent willingness to abandon this idea in his 4th comment is sound, but his alternate suggestion does not provide a satisfactory manner for driving phosphorylation by protonation.

It must be emphasized that my criticisms of Mitchell's suggestions for how a membrane potential and/or a proton gradient might be used for ATP synthesis are not directed towards his outstanding contributions demonstrating formation of potential and of H^+ gradients across the mitochondrial inner membrane and his splendid support of the concept that such potential and H^+ gradients can be used reversibly for ATP formation and for active transport.

(Subsequent to submission of this paper, a recent contribution of R. J. P. Williams, FEBS Lett., 53, 123–125, came to the author's attention. In his paper Professor Williams calls attention to deficiencies in the 'chemiosmotic molecular mechanism' for ATP synthesis suggested by Mitchell.)

energy input also serves to increase affinity for P_i and/or ADP, with the interconversion of bound ADP and P_i to ATP as the step least sensitive to uncouplers [12].

The principal purpose of this paper is to point out how energy-linked conformational changes provide an attractive means for transducing energy of a membrane potential or a proton gradient to the high-energy phosphate of ATP. The suggestions are also applicable to some ATP-linked active transport processes.

The model, although deceptively simple, does not appear to have been presented previously, and readily explains a fixed stoichiometry of proton translocation coupled to ATP formation as well as a summation of membrane potential and proton gradient to drive ATP formation.

2. Charged group migration as a key event in conformational coupling

There is impressive evidence that a negative potential and higher pH in the mitochondrial matrix space [2,3] in the chloroplast intermembrane space [4] or inside bacterial cells [5] can promote ATP formation or be induced by ATP cleavage. A membrane potential can favor movement of a charged group from contact with solvent water on one side of the membrane to the other side**. For example, with mitochondria a positively charged group might be induced to move into the matrix space or a negatively charged group might be induced to depart from the matrix space. A net proton movement may result from return of the uncharged forms. The protonation of the participating groups gives a means by which proton gradients across the membrane can assist in the migration. If the group migration were reversibly linked through protein conformational changes to the events leading to formation or utilization of ATP at the catalytic site, energy-linked coupling of the potential to ATP synthesis or of ATP cleavage to proton translocation and potential generation would result.

The diagram in fig.1 extends and helps illustrate

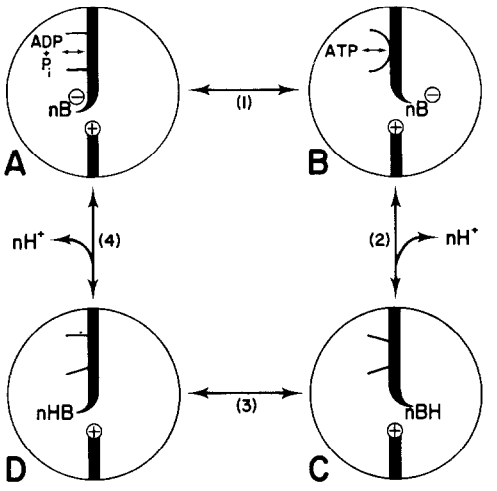

Fig.1. Diagrammatic sketch of conformational coupling of phosphorylation to membrane potential or proton gradient. $-B^{\ominus}$ represents a proton accepting group, a number, n, of which change exposure from the one side to the other of a membrane coupled to conformation and catalytic changes at the phosphorylation site that drive ATP synthesis from ADP and P_i. A positive charge $\oplus$ is depicted at a membrane pore that prevents $H_3O^{\oplus}$ diffusion but allows changes in exposure of $B^{\ominus}$ and free diffusion of H_2O.

Changes in binding and catalytic properties of the phosphorylation site are depicted by transitions from ⊐,),] and] in stages A, B, C and D respectively. The conversion A → B → C → D through steps 1 to 4 respectively serves to drive ATP synthesis coupled to translocation of n protons across the membrane. Operation of the cycle in the opposite direction (D → C → B → A) would couple translocation of n protons across the membrane coupled to ATP cleavage.

the above possibilities. Two functional sites are indicated in the figure, the phosphorylation site for ATP cleavage and synthesis and a site undergoing reversible protonation involved in proton translocation. Four intermediate states, A, B, C, and D, are illustrated, but additional intermediary states must also intervene. For example, events of ATP, ADP and P_i binding and release as well as interconversion are not shown. For ATP synthesis coupled to proton translocation the transitions would occur through steps 1,2,3 and 4. This would be favored by a negative potential and/or higher pH in the left space (the matrix space in the mitochondria). Operation in the reverse direction would couple ATP cleavage to proton transport. This would be favored by a smaller negative or a positive potential and/or a lower pH in the left space.

** In this paper movement of a charged group is indicated, but it should be recognized that a change in position of membrane groups shielding the charged group from solvent would be equally plausible.

For reasons indicated below, a negatively charged group, B^-, is considered to be a likely migratory group. A protein carboxyl group could quite conceivably be involved. For ATP synthesis, the key transition is from state A to state B. For example, in mitochondria, the negative potential in the matrix space is visualized as favoring the movement of the B^- group to the opposite side of the membrane. This movement may be conformationally linked to ADP and P_i binding and ATP formation and release. With mitochondria, a low H^+ activity inside the matrix as compared to the exterior would promote ATP synthesis by making more of the B^- form available on the interior.

For proton translocation coupled to ATP cleavage, the binding of ATP in state B is regarded as inducing conformational change favoring movement of the B^- group from the right to the left side of the membrane (fig.1) thus increasing the negative potential on the left side. The change from state B to state A includes cleavage of ATP to ADP and P_i and release of products. The B^-group on the left side will be protonated, to the extent determined by the pH, to give state D. The protonated, uncharged group can readily migrate back from left to right through the membrane with formation of state C. The dissociation of the BH group to convert state C to state B results in net proton translocation across the membrane.

The interconversions between each of the 4 states is regarded as resulting in small but vital conformational changes at the phosphorylation catalytic site. These are crudely depicted in the figure by small changes in the shape of the site. The events linked to the conformational changes include changes in affinities of reactants at the catalytic site and modifications in catalytic capacity of the phosphorylation site. The phosphorylation site, for example, must allow ATP cleavage coupled to movement of the H-binding group only when the group is negatively charged and not when it is protonated. Recognition by the ATP site of whether or not the transducing group is protonated provides a simple means of providing the vectorial component necessary for any energy-linked transport.

3. Possible energy-linked movement of a positively charged group

A similar series of events may be considered for movement of a positively charged group, but with some distinct differences. Other means would need to be provided to prevent H_3O^+ migration through the incipient channel. In state A the conformations for mitochondria would be such that the positively charged group is exposed to the opposite side of the phosphorylation site. The membrane potential, negative in the matrix space of mitochondria, would thus favor the movement of the $-BH^+$ group to the interior, coupled to the conformational transitions leading to ATP synthesis. Conversely, with ATP cleavage a transition from state B to state A would, by withdrawing a positive charge, add to the negative potential inside the matrix. Dissociation of the proton in the intermembrane space would give the uncharged group that could migrate back to the interior. Protonation of this group, and its expulsion coupled to ATP cleavage would complete a cycle of proton translocation coupled to ATP cleavage.

4. The stoichiometry of the conformational coupling

In fig.1 an indeterminate number, n, of charged groups is depicted as being coupled to the cleavage of one ATP molecule. Data of Mitchell and Moyle suggest that at least two protons can be translocated for every ATP cleaved [13] and other results have suggested higher equivalents of H^+ or K^+ to ATP [14–16]. Higher H^+/ATP ratios can readily be accommodated by the model, indeed this is one of the attractive features. A protein subunit in a membrane might have several charged groups aligned in the plane of the membrane such that a small movement of the entire subunit would change the exposure from the inner to outer aqueous phase.

An important stoichiometric feature is that the model requires the movement of a fixed number, n, of protons to be transported for each ATP molecule synthesized or used. If the B^- group can move only when coupled to ATP synthesis or cleavage, a weak membrane potential and/or proton gradient would diminish the extent or rate of the coupled processes but the stoichiometry would remain the same.

5. A distinction from models based on conformationally-induced changes in p*K*

Electron transport coupled to proton uptake and

release by mitochondrial and chloroplast membranes may involve a change in the pK of membrane groups, or a 'membrane Bohr effect' [17]. Such changes in pK have been discussed as a means of linking proton transport to oxidations and phosphorylations.

The simple suggestion of conformational coupling to proton transport through changes in pK does not readily explain such features as a fixed stoichiometry of proton transfer to ATP formation, a net proton movement in repeated cycles, or the use of membrane potential as a driving force for ATP synthesis. Suitable explanation might be provided by amplification of the suggestion to include features of the model depicted in fig.1. But then the coupled charged group migration and not the change in pK would be vital. It is of course plausible that the B^- group depicted in fig.1 might have a different pK when exposed to opposite sides of the membrane, but such a change in pK is not an essential part of the coupling mechanism.

6. Membrane potential or proton gradient as the primary driving force

The suggested coupling mechanism could under some circumstances involve only membrane potential as the primary driving force. Such potential might be generated by K^+ ion migration in presence of valinomycin under suitable conditions as well as by proton translocation. Oxidatively induced proton translocation with buffered media of equal pH on both sides of the membrane would still establish a membrane potential. However, as noted in the above discussion, a pH gradient could promote a distribution of the migrating group so as to favor ATP synthesis. An H^+ gradient may play a more important role in chloroplasts than in mitochondria [18]. The protein and change arrangement may be such that the group B^- once formed by deprotonation tends to move to the opposite side. Proton removal from the BH form would thus provide an important means to drive the cycle. The scheme is thus adaptable to the possibility of ATP synthesis driven by proton gradients even in the absence of a membrane potential.

Because either a membrane potential or a proton gradient or both could drive ATP synthesis, the scheme is quite compatible with Mitchell's valuable suggestion of the addition of potential and proton gradients (the 'protomotive' force) to give the total energy available for ATP synthesis. But my proposal is not compatible with Mitchell's chemiosmotic coupling mechanism for use of membrane potential and proton gradients for ATP synthesis [7,19], where a migrating proton is regarded as having increased activity usable in some manner for covalent bond synthesis. In a scheme such as presented here protons behave in a normal manner in an equilibrium protonation of basic groups depending on the pH of the surrounding medium.

7. Some molecular characteristics of the transduction

The requisite small magnitude of the conformational changes deserves emphasis. Small movements, even if against a considerable energy barrier, allow efficient energy transduction. If large movements occur, the accompanying molecular reorganization could dissipate energy. Merely for clarity, fig.1 depicts changes that might be taken to imply movements of a group for a considerable distance and major conformational changes. Changes in affinities of reactants at a catalytic site can readily occur with only subtle changes in the Ångström range. The electrogenic migrations of the charged group could transmit the requisite change principally through positional shift of a membrane protein subunit without any major conformational transition. No 'flip-flops' or protein rotations need be involved. The only transition required for the charged group is a relocation so as to have access to solvent water and protons on either side of the membrane. As noted below, water molecules might freely cross the membrane at the transducing site, although this is not vital to the model.

An important requirement might be that the migrating charged group does not move through a space of low dielectric constant such as a hydrophobic portion of the membrane. There is a considerable energy barrier for the desolvation and isolation of the charged group that would be required. It appears more attractive for water to have free access across the transducing site, but with some means of blocking the migration of cations, including protons, through the site. This could readily be achieved by appropriate location of a positive charge or charges. Such a charge is included in the scheme of fig.1. This illustrates one way of meeting the important requirement of maintenance of the negative membrane potential but with water environment for the migrating group.

The likelihood of a positive charge at the transducing pore favors a negatively charged group as the migrating group for the energy transductions. Indeed, the migrating group might have a transitory ionic attraction to the positively charged group during the transition from one state to another but there would be little energy barrier for the key group migration. Other solvent anions could likewise have sufficient access to the positive membrane group to avoid charge isolation. The aqueous pore at the transducing site might well serve as a port of entry and exit for some permeant anions.

8. The coupling to active transport

A series of experiments stimulated by the concepts of Mitchell have given convincing demonstration that potential gradients can be coupled to transport of solutes. The experiments of West and Mitchell [20] demonstrating coupling of lactose transport in *E. coli* to a membrane potential are an excellent example. Obvious extensions of the model depicted in fig.1 could apply to active transport of various metabolites. For example, the charged group translocation could be linked to conformational changes that decrease the apparent affinity of the transported ligand for its binding site at the inner membrane surface.

Only small changes in membrane structure and associated conformational changes need accompany change in access of a bound ligand from the outer to inner aqueous phase. Change in binding of the ligand at the inner membrane phase could result from a higher K_d for binding, see [21], but the possibility of coupling by energy-linked blocking of access to the site once the ligand has dissociated [22] also merits consideration.

Coupling of energy to transport of Ca^{2+}, K^+ and other ions can similarly be readily accommodated by the present model. For bacterial transport systems, where the transport system and the oxidative or ATP energy sources are located in the same membrane, an electrogenic mechanism may operate. Either oxidation or ATP cleavage could create the requisite membrane potential. This could be used to change affinities of ions at different sites of the membrane, similar to changes in affinity for substrates as depicted for ATP synthesis in fig.1. Again, stoichiometry of more than one ion transported per unit charge dissipated can readily be accommodated. Such a mechanism has additional appeal. It would mean that Nature is using a molecular machine of similar design for both ATP synthesis and active transport.

The specialized transport ATPases such as the microsomal Na^+, K^+-ATPase and the Ca^{2+}, Mg^{2+}-ATPase of the sarcoplasmic reticulum probably function through direct conformational coupling within the membrane ATPase rather than indirectly through membrane potential. For this, the conformational changes accompanying ATP binding and cleavage could be transmitted directly to the affinity sites for ions. A very small change in position of liganding groups could change binding preference from Na^+ to K^+, or from Ca^{2+} to Mg^{2+}. Such changes, accompanied by the minimal transitions necessary for exposure to the aqueous phase on either side of the membrane, would complete the transport process.

The above suggestions for coupling by transport ATPases are in contrast to the suggestions of Mitchell [23] that complexes of ATP and its hydrolysis products bind to the transported ions, and that the water oxygen for ATP cleavage comes from the opposite side of the membrane from the ATP. My suggestions are consistent with and in part derived from other current concepts of membrane structure and transport mechanisms (see Singer, [24]).

9. Coupling of oxidation to ATP synthesis

Brief comment may be appropriate on relationships of conformational coupling as presented here to synthesis of ATP coupled to electron transport by mitochondria and chloroplasts. As mentioned previously, involvement of electrical potential or proton gradients in transmission of energy from oxidations to the phosphorylation complex is regarded as an attractive possibility. But in one sense both proton translocation and protein conformational change could operate in the energy transmissions of oxidative phosphorylation and photophosphorylation. Energy-requiring conformational changes initiated at the sites of electron transfer could be transmitted through protein structures to sites for proton translocation, then transmission to the phosphorylation complexes completed by the potential or proton gradients produced.

Also attractive at this stage is the possibility of direct conformational transfer of energy from electron transport to ATP synthesis in oxidative phosphorylation and photophosphorylation [25]. Indeed, direct conformational interactions appear probable in the ATP-requiring electron transfer reactions occurring in nitrogenase [26].

10. Concluding statement

Acceptance of a membrane potential and/or a proton gradient as a possible means of transmitting energy from oxidations to ATP synthesis rests in part on a satisfactory hypothesis for how the potential or proton gradient could drive ATP synthesis. Recognition that energy input may drive ATP synthesis by change in binding of reactants at the catalytic site has led to the suggestions presented in this paper. These are that in oxidative phosphorylation and photophosphorylation, the requisite conformational changes may be coupled to exposure of charged groups to different sides of the membrane. The cycle of charged group exposure or movement may be driven by the membrane potential or, through protonation and deprotonation, may be coupled to proton translocation across the membrane. Effects of proton gradient and membrane potential may be additive. Similar conformational coupling suggestions may explain proton translocation coupled to ATP cleavage and active transport of metabolites coupled to membrane potential, proton gradients of ATP cleavage.

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